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Life cycle of *Haematoloechus similis* (Looss, 1899) *(Trematoda — Plagiorchiidae)*

Rozwój *Haematoloechus similis* (Looss, 1899) *(Trematoda — Plagiorchiidae)*

Trematodes of the genus *Haematoloechus* Looss, 1899, are common and known for a long time parasites of amphibia, but their life cycles with the exception of several species have not been established. Relatively best known are the life cycles of species occurring in North America (Krull 1932—1933, Ingles 1933, Rankin 1939). The European species were originally investigated by Sinicyn, 1905. He found a metacercaria in the body cavity of a dragonfly, *Calopteryx virgo,* identified it as *H. variegatus,* and then fed it to frogs. More detailed studies on the development of *H. variegatus* were undertaken by van Thiel, 1930 who obtained results quite different from those of Sinicyn. Van Thiel discovered that cercariae of this species occur in *Planorbis vortex (C. anophelis* van Thiel, 1924) and are very similar to *C. prima* Sinicyn, 1905. They penetrate the larvae of *Anopheles maculipennis* forming metacercariae *(Agamodistomum anophelis* van Thiel, 1924) completely different from those described by Sinicyn. Recently Genecinskaja (1959) found *C. prima* in Rybinski Lake in the USSR and assumed it to belong to *H. similis.*

Outside of these slightly inconsistent data, the life cycles of trematodes of the genus *Haematoloechus* occuring in Europe remained unknown.

While examining in the spring of 1958 in the vicinity od Warszawa larvae of *Odonata,* the author found in dragonflies of the genus *Coenagrion* Kir. *(Zygoptera)* matecercariae identical to those described by Sinicyn as *H. variegatus.* Simultaneously snails,

Planorbis planorbis (L.), were found to contain cercariae which penetrated the dragonflies giving identical metacercariae. On the basis of morphology of metacercariae and results of experimental infestation of frogs, the metacercariae were identified as *Haematoloechus similis* (Looss, 1899). This was further ascertained by invading snails with eggs of this fluke species.

Since metacercarie described by Sinicyn did not differ from the above, they should be considered to belong to the species *H. similis*. Sinicyn described them on the basis of Looss' work published in 1894 (Die Distomen unserer Fische und Frösche) in which *H. similis* was not considered as a separate species.

The first experiment was performed in 1958 by invading 6 young dragonflies, *Coenagrion hastulatum* Charp., with cercariae found in naturally invaded specimens of *Planorbis planorbis*. The dragonflies were examined every few days in the course of three weeks. All of them contained various numbers of developing metacercariae, namely: 4, 14, 4, 18, 25 and 17. The metacercariae did not encyst and moved about freely in the body cavity of the dragonfly. After three weeks they differed from the trematodes found in natural invasion only in their smaller size. In natural invasion no dragonfly was found to contain more than 3 metacercariae.

The investigation was continued the following year. 76 dragonflies of the genus *Coenagrion*, taken from a pond in which no cercariae or metacercariae were found, were invaded with cercariae of *H. similis*. The dragonflies were invaded individually, in a small amount of water. Each was allotted 15 to 20 cercariae. After 5 hours about 2/3 of the cercariae penetrated into the dragonflies. The development of metacercariae was not identical in all the *Odonata*. In *Coenagrion hastulatum* Charp. and *C. armatum* Charp. the development proceeded normally and after a month 4 to 6 mature metacercariae developed in each specimen. In *C. pulchellum* V. d. L., however, the majority of metacercariae were found dead already on the second day following infestation and none could be found after several more days. A final positive result was obtain in only 15 cases of this series.

The young specimens of *Rana esculenta*, 9 in number, were subsequently invaded with these metaceriariae. The frogs were about six months old and were kept in terrarium since metamorphosis under conditions excluding the possibility of infestation. Each frog received 1 or 2 metacercariae which together with pieces of dragon-

flies were slipped into their oesophagus. After 40 to 60 days the frogs were examined. The lungs of 4 frogs were found to harbour one specimen of *H. similis* each.

In order to ascertain the results obtained, 10 specimens of *Planorbis planorbis* reared from eggs laid in an aquarium were invaded with eggs of *H. similis*. The snails were placed together with fluke eggs in a small Petri dish containing a small amount of water. After 24 hours their feces contained a large number of empty egg shells. The snails were examined after 40 days and all were

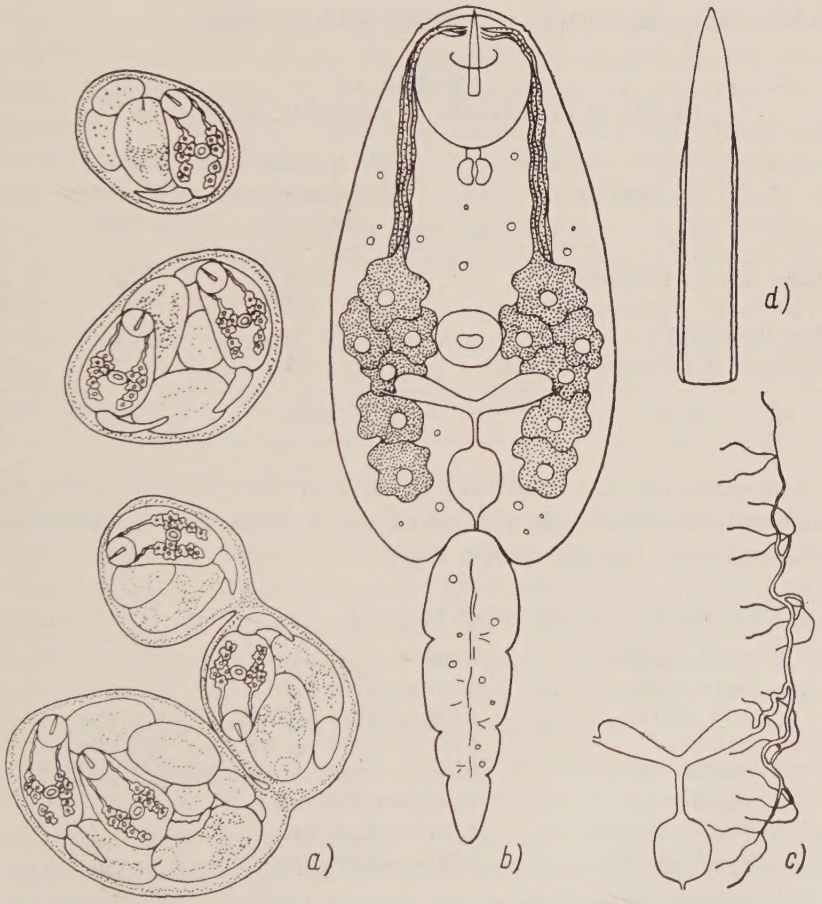

Fig. 1. *H. similis* (Looss): a — sporocysts, b — cercaria, c — excretory system of the cercaria, d — stylet.

found to harbour sporocysts and a certain number of mature cercariae identical to those used to invade the dragonflies.

Sporocysts (Fig. 1a). The sporocysts are small bags of spherical or oval shape. They often consist of several parts, usually 2 or 3, connected with one another by thin tubings without lumina. Each sporocyst contains 1 to 3 mature cercariae. The measurements of the sporocysts are 0.163 to 0.600 by 0.132 to 0.273 mm.

Cercariae (Fig. 1b). Cercariae in natural invasion were found in Lake Mamry Północne, in the vicinity od Warszawa and in the coastal region. They usually occurred in small bodies of water. At Lake Mamry nearly all the infected snails were found in a small muddle only periodically connected with the lake.

Table I

Results of examination of *Planorbis planorbis* (L.)

Locality	Number of specimens examined	Number of specimens invaded	%
Lake Mamry Północne	3058	35	1.1
Vicinity of Warszawa (Julin)	355	14	3.9
Hel (Kuźnica)	75	7	9.3
Vicinity of Lake Żarnowieckie	35	9	25.7
Total	3523	65	1.8

The cercariae are relatively small and very mobile. They swim persistently in the whole volume of water displaying no geotropism, and sometimes, though rarely, creep.

Dimensions of cercariae (killed by heat)

body length	0.130 — 0.164 mm
body width	0.087 — 0.104 mm
oral sucker	0.034 — 0.042 by 0.037 — 0.046 mm
pharynx	0.011 — 0.013 by 0.015 — 0.017 mm
ventral sucker	0.020 — 0.022 by 0.020 — 0.024 mm
stylet	0.032 — 0.034 mm
length of tail	0.102 — 0.136 mm
width of tail	0.027 — 0.034 mm

The cuticle is smooth on the whole body showing no trace of spines. At the anterior end and laterally to the oral sucker there are two pairs of papillae. No cilia were noted on the surface of the

body. The tail, comparatively short but well musculated and highly contratile projects from a small invagination. There are no caudal pockets and no finfold. The ventral sucker equals in size 1/2 the oral sucker and is situated in the centre of the body. The parenchyma of cercariae is completely transparent as it does not contain any cystogenous glands.

In the oral sucker there is a stylet, very large in comparison to the size of the body. It is slender and sharply pointed with nearly parallel margins and no transverse thickening. The penetration glands, 6 pairs in number, are large and very pronounced. They are situated along the lateral margins of the body begining anteriorly to the ventral sucker and extending to the posterior end of the body. They form two groups, the anterior one consisting of 4 glands and the posterior one of 2. The two groups are separated by lateral branches of the excretory vesicle. The glands are filled with a finely granular fluid substance and their shape changes with contractions of the body. They are usually star shaped. The nuclei of gland cells are large and easily observable. The digestive tract is not developed and only a slightly differentiated pharynx preceded by a very short praepharynx is observable.

The excretory system is hardly apparent since the excretory canals are very thin. The vesicle is Y shaped and opens outwards at the base of the tail. No canal appears to run through the tail. The flame formula is $2\ [(3 + 3 + 3) + (3 + 3 + 3)] = 36$. The flame cells are very small and inconspicuous.

Cercaria of *H. similis* displays features characteristic of two groups of xiphidiocercariae. On the one hand, its size, body proportions and structure of sporocyst shows resemblance to the „prima" group of *Cercariae ornatae,* while on the other, its excretory system and lack of the caudal finfold resemble *C. armatae.* The features which make it differ from both these groups are a system of penetration glands and lack of cystogenous glands.

M e t a c e r c a r i a e (Fig. 2). Having found the proper host, the cercaria creeps on it for some time in search of a suitable place to pierce the shield. It usually penetrates it at the base of the head or appendages, more rarely between segments of the abdomen. It penetrates quickly, within several odd minutes, throwing away the tail in the meantime. It then passes through a muscle layer into the body cavity.

The young metacercariae are highly mobile for several hours after penetration and creep inside the dragonfly searching for a most suitable place. Afterward they gather in the anterior part of the abdomen where they remain nearly motionless, but do not encyst. During the first few days following infestation a large number of them perishes. In the surviving ones the penetration glands disappear and after several days the stylet disintegrates, but for some time its vestiges may be found in the oral sucker. Subsequently develop the branches of the excretory vesicle. Inside the vesicle appear small excretory granules. After about two weeks the digestive tract develops and spines appear on the cuticle. The metacercariae reach their maximum size in one month.

Dimensions (in mounted specimens)

body length	1.990 — 2.290 mm
body width	0.575 — 0.671 mm
oral sucker	0.163 — 0.184 by 0.180 — 0.201 mm
pharynx	0.073 — 0.086 by 0.090 — 0.104 mm
ventral sucker	0.14 — 0.135 by 0.118 — 0.145 mm
ovary	0.080 — 0.107 by 0.062 — 0.097 mm
receptaculum seminis	0.062 — 0.076 by 0.052 — 0.083 mm
anterior testis	0.132 — 0.180 by 0.125 — 0.152 mm
posterior testis	0.152 — 0.173 by 0.125 — 0.142 mm

The cuticle of mature metacercariae is covered on the whole surface with minute but easily to distinguish spines. The parenchyma is not transparent, making the trematodes appear white. The ventral sucker grows more quickly than the oral one reaching a size equal to 2/3 of the oral one similarly as in mature worms.

The digestive tract is well developed. Immediately behind the oral sucker there is the pharynx. The esophagus is very short and caeca extend to the end of the body. They are often filled with a greenish substance probably derived from the body cavity of the dragonfly.

A characteristic feature of the metacercariae is a well developed excretory vesicle, the main trunk of which bifurcates slightly below the ventral sucker to form two wide undulating canals extending to the oral sucker. The excretory granules are minute and do not fill the entire cavity of the vesicle. They probably remain inside the cells forming the walls of the vesicle.

In mature metacercariae the reproductive organs are already developed and located similarly as in adult trematodes.

The ovary is situated at the left side near the ventral sucker. Below there is a small empty vesicle — receptaculum seminis. Beside the receptaculum the anlage of the ovary may be observed although the ootype and the Mehlis glands are not visible. The uterus forms a straight loop and its descending branch extends only to the end of the intestine where it turns backwards. The ascending branch extends along the median line to the genital pore situated at the

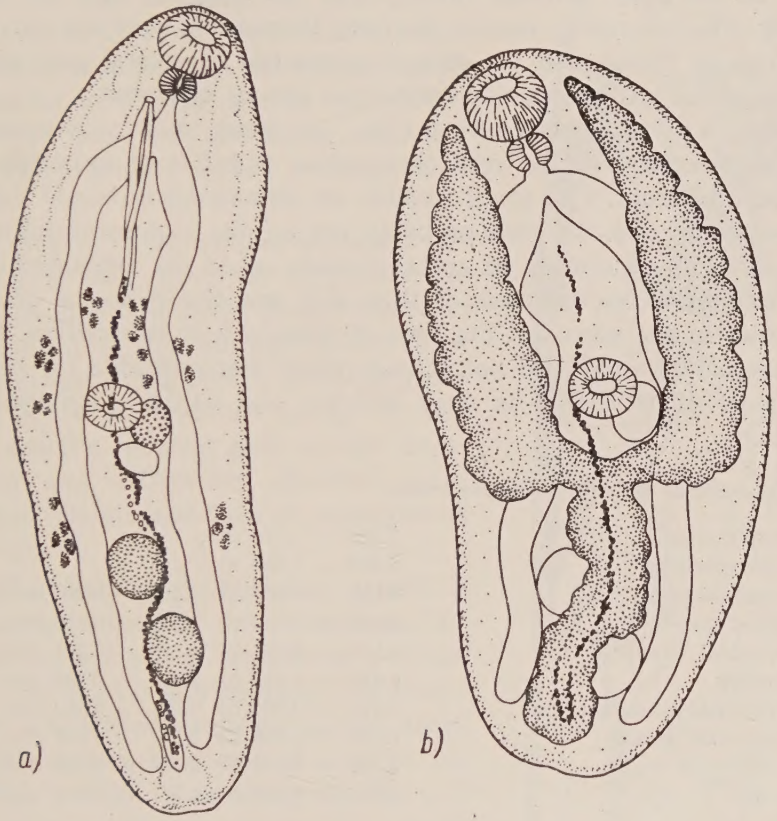

Fig. 2. *H. similis* (Looss): a — metacercaria stained with carmin alum, b — living metacercaria.

right side near the pharynx. In older metacercariae the uterus is filled with round or oval dark brown bodies measuring from 3 to 17 μ. Similar bodies are often found in uterus of young trematodes belonging to the genus *Haematoloechus* and their appearance usually precedes production of eggs.

The vitellaria arranged in rosettes form two clusters at the margins of the body. Those anterior to the ventral sucker are better developed and consist of 2 or 3 rosettes extending to the median line over the intestine. Below the ventral sucker there are 1 or 2 rosettes lying outside of the intestine. They extend only to the anterior testis.

Oval nearly spherical testes are placed obliquely in the posterior half of the body between branches of the intestine and the main trunk of the excretory vesicle. In living trematodes they are partially covered by the vesicle. On stained specimens the distal part of the cirrus pouch lying alongside the uterus can be observed.

Mature trematodes (Fig. 3). Four mature worms of *Haematoloechus similis* were obtained as a result of experimental invasion of frogs. The small number of trematodes obtained can be explained by the fact that probably not all the metacercariae were invasive. The maturity of metacercariae could be estimated only approximately on the basis of their size and the presence of dark granules in the uterus at the time of dissection of dragonflies. Only metacercariae which did not contact water were suitable for infection since their cuticle is very delicate and rapidly damaged by water.

Dimensions (in mounted specimens)

body length	2.730 — 4.770 mm
body width	0.760 — 1.060 mm
oral sucker	0.194 — 0.280 by 0.201 — 0.302 mm
pharynx	0.086 — 0.110 by 0.125 — 0.140 mm
ventral sucker	0.135 — 0.208 by 0.125 — 0.200 mm
ovary	0.225 — 0.420 by 0.163 — 0.354 mm
receptaculum seminis	0.243 — 0.384 by 0.156 — 0.310 mm
anterior testis	0.191 — 0.369 by 0.163 — 0.354 mm
posterior testis	0.253 — 0.443 by 0.180 — 0.361 mm
eggs	0.031 — 0.033 by 0.017 — 0.019 mm

The youngest of the trematodes obtained after 40 days from the time of infestation had a uterus filled with eggs smaller than normal having a light and soft shell. The lateral loops of the uterus were just beginning to form. The remaining 3 worms obtained after 50 and 60 days were already mature. The uterus had already developed its lateral loops and the eggs were dark brown, of normal dimensions. They contained developed miracidia.

When comparing the development of *Haematoloechus similis* with the known life cycles of other related species, marked differences in the morphology of cercariae and metacercariae become apparent.

Certain characteristic features of cercariae of species occurring in North America and also of *H. variegatus* entitle them to be included in the group „prima" of *Cercariae ornatae*. The main characteristic feature of this group is a finfold at the end of the tail. Other features are less definitive and the excretory system awaits detailed analysis.

Cercaria of *H. similis* although resembling this group of xiphidiocercariae in size and body proportions has no finfold on the tail and its excretory system resembles that of cercariae from the *Cercariae armatae* group. It also has specific features such as well developed and differently situated penetration glands and lack of cystogenous glands.

The lack of cystogenous glands is in a certain sense detrimental to the development of the metacercaria which does not encyst and its growth is therefore not limited. In other species encystation occurs as a rule at least in the initial period. According to Krull encysted metacercariae reach a maximum body length of 0.350 to 0.410 mm while in *Pneumobites longiplexus*

Fig. 3. *H. similis* (Looss), mature trematode obtained 60 days following infestation.

(Stafford), which encysts in the initial period only, the metacercariae reach a length of 0.900 mm. Metacercariae of *H. similis* are enormous in comparison with the encysting ones reaching the length of over 2 mm. Their development, however, takes much longer. Metacercariae of *H. similis* are also more than other species advanced in respect to differentiation of reproductive organs.

Summary

The author has established experimentally the life cycle of *Haematoloechus similis* (Looss, 1899). The first intermediate host has been found to be *Planorbis planorbis* (L.), the second intermediate host were species of dragonflies of the genus *Coenagrion* Kir. Developmental stages have been described and differences between the development of *H. similis* and related species discussed.

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STRESZCZENIE

Autorka przeprowadziła eksperymentalnie rozwój przywry *Haematoloechus similis* (Looss, 1899) występującej w płucach żaby *Rana esculenta* L.

Pierwszym żywicielem pośrednim *H. similis* jest ślimak *Planorbis planorbis* (L.), w którego wątrobie rozwijają się małe woreczkowate sporocysty zawierające niewielką ilość cerkarii. Cerkarie mają charakterystyczny prosty i długi sztylet oraz gruczoły penetracyjne położone wzdłuż boków ciała. Nie mają gruczołów cystogennych. Cerkarie były znalezione w zarażeniu naturalnym u 1,8% zbadanych *Planorbis planorbis* oraz wyhodowane eksperymentalnie z jaj *H. similis*.

Drugim żywicielem pośrednim są ważki z rodzaju *Coenagrion* Kir. Metacerkarie nie wytwarzające otoczki przebywają w jamie ciała ważek osiągając znaczne rozmiary. Dojrzałe metacerkarie mają wysoko zróżnicowane narządy rozrodcze, położone tak samo jak u przywr dorosłych tego gatunku.

Pod względem budowy cerkaria i metacerkaria *H. similis* różni się dosyć znacznie od pokrewnych gatunków, których rozwój został poznany.

Na podstawie analizy morfologicznej autorka stwierdziła, że opisana przez Sinicyna w 1905 r. metacerkaria *H. variegatus* należy do gatunku *H. similis*.